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09/662,462	09/15/2000	Terry Smith	2551-49	3620

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NIXON & VANDERHYE P C
1100 North Glebe Road
8th Floor
Arlington, VA 22201-4714

EXAMINER
GOLDBERG, JEANINE ANNE

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1634	24

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/662,462	Applicant(s) SMITH ET AL.	
	Examiner Jeanine A Goldberg	Art Unit 1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *24 June 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *41-63* is/are pending in the application.

4a) Of the above claim(s) *53* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *41-52 and 54-63* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. This action is in response to the papers filed January 10, 2002. Currently, claims 41-63 are pending. All arguments have been thoroughly reviewed but are deemed non-persuasive for the reasons which follow.

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on June 24, 2002 has been entered.

3. Any objections and rejections not reiterated below are hereby withdrawn.

4. All previously pending claims have been cancelled in favor of newly submitted Claims 41-63, therefore, the previous grounds of rejection have been withdrawn and the following new grounds of rejection are applied.

Election/Restrictions

5. Newly submitted claim 53 is directed to an invention that is independent or distinct from the invention originally claimed for the following reasons.

Claim 53 is directed to withdrawn subject matter of Groups II-IV set forth in Paper Number 10, April 10, 2001. The claim is drawn to SEQ ID NO: 14-32, 39-43 which are non-elected sequences.

Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for

prosecution on the merits. Accordingly, claim 53 is withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 41-52, 54-63 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A1) Claim 41-52, 54-63 are indefinite because Claim 41 is drawn to "an isolated oligonucleotide molecule" however the claim further recites "wherein all said oligonucleotide sequences". Therefore, it is unclear whether the claim is drawn to a single oligonucleotide or whether the claim is drawn to the set comprising all of the oligonucleotides. Furthermore, the claim is drawn to "wherein all said oligonucleotide sequences are functional under identical hybridization conditions". It is unclear how "an oligonucleotide" may not be functional with itself. Therefore, it does not appear that such a limitation is afforded any weight in the instant claim. Moreover, "functional" is indefinite because it is unclear what function the sequences must have. The sequences are all functional under identical hybridization conditions to either remain in solution or hybridize to target nucleic acids. Moreover, depending upon the hybridization conditions, low stringent conditions would allow all of the oligonucleotides to hybridize to sample DNA. Therefore, the metes and bounds of the claimed invention are unclear.

B1) Claim 45-52, 56-63 are indefinite over the recitation "wherein the at least one probe of step (iii)" because step (iii) of Claim 43 does not refer to a probe. Claim 43, 54 refers to "at least one of the oligonucleotide sequences of Claim 41".

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Newly added Claims 41-43, 45, 47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Botelho et al. (Yeast, Vol. 10, pg. 709-717, 1994) in view of Hogan (U.S. Pat. 5,595,874, January 1997).

Botelho et al (herein referred to as Botelho) teaches specific identification of *Candida albicans* by hybridization with oligonucleotides derived from ribosomal DNA internal spacers. Botelho teaches an alignment of *Candida albicans* and *Candida tropicalis*. Botelho teaches that the ITS1 and ITS2 regions were found to contain distinct regions with sufficient sequence divergence to make them suitable as specific target sites for the identification of *C. albicans*. Botelho teaches that comparison of the ITS sequences was performed by computer-aided sequence comparison using the software SEQNCE and FASTA to find optimal sequence alignment. Regions of variability were highlighted by the computer which were ideal regions to probe. These regions were ANAB2 and ANAB3. Botelho teaches that the spaces showed sufficient sequence divergence to allow design of species-specific probes (page 715, col. 1).

SEQ ID NO: 1, 2, 3, 6, 36 are embedded within the ITS1 region. The sequences are found in regions of variability. Botelho teaches detecting and identifying fungal pathogenic species in a sample by releasing the nucleic acids of the pathogens, amplifying the ITS with a fungal universal primer pair, hybridizing the nucleic acids with a species specific oligonucleotide probe, and detecting the complexes formed (pg. 714-715). Botelho teaches that the probes which were identified unequivocally distinguish between *C. albicans* and other yeast generas as well as between .*C albicans* and other medically important Candida strains such that the have great potential as diagnostic tools (pg. 715, col. 2). Botelho teaches that the ITS1 and ITS2 regions have low interspecies homology which makes them ideal probes to differentiate species.

Botelho does not specifically teaches the exact probes and primers of the instant application.

However, Hogan et al. (herein referred to as Hogan) teaches variable regions can be identified by comparative analysis of sequences by using a Sun Microsystem ™ computer for comparative analysis. The "compiler is capable of manipulating many sequences of data at the same time. Computers of this type and computer programs which may be used or adapted for the purposes are commercially available" (col. 5, lines 50-60). Hogan also teaches the use of specific primers col. 6-7, lines 50-67, lines 1-12, and furthermore provides specific guidance for the selection of primers and probes.

> "Once the variable regions are identified, the sequences are aligned to reveal areas of maximum homology or 'match'. At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.
> First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some

> others). Second, the stability of the probe:target nucleic acid hybrid should be maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10^{o}C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less preferred. Finally, probes with extensive self complementarity should be avoided."

Therefore, it would have been **prima facie** obvious to one of ordinary skill in the art at the time the invention was made to have designed probes and primers to *Candida albicans* and *tropicalis*, as taught by Botehlo, to the regions of variability found between the two species such that the two species may be differentiated as taught by Hogan. The skilled artisan would have been motivated to have designed probes and primers to these *Candida albicans* and *tropicalis* species for the benefit of differentiating the species from one another. Botelho teaches that the probes which were identified unequivocally distinguish between *C. albicans* and other yeast generas as well as between *C albicans* and other medically important Candida strains such that the have great potential as diagnostic tools. Thus, the ordinary artisan would have been specifically motivated to identify and differentiate the Candida species. The knowledge in that art at the time the invention was made was extremely high for aligning sequences and finding regions of variability such that sequence comparison and differentiation between internal spacer regions to identify regions of either variability or conservation was well known and studied. The art teaches an alignment of known sequences, namely *Candida albicans,* and *Candida tropicalis,* as taught by Botelho. Identifying regions of variability between the two species to generate probes which are species specific is as taught by Hogan. Within the alignment provided between a select group of the Candida species provided by Botelho, the instant probes are within regions of variability. The ordinary artisan would have been motivated to have designed probes and primers to the region of variability of the ITS1 region to detect either *C.*

albicans or *C. tropicals*. Thus, in view of the teachings in the art, a method for detecting fungal pathogenic species using the probes of SEQ ID NO: 1-3, 6, 33, 36 are obvious.

Response to Arguments

The response traverses the rejection. The response asserts, at page 15, that "the Examiner must also appreciate that Botelho must use different hybridization conditions for its probes". This argument has been thoroughly considered but is not convincing because the single oligonucleotides, Claim 41, have the functionality under identical hybridization conditions. Moreover, detection of one Candida species in one single assay using one oligonucleotide, Claim 43, also is performed under a single hybridization condition. Therefore, Claim 42-52 are not drawn to using more than one probe in a single assay, nor does the claim have any recitation of "under the same hybridization conditions" which applicants appear to consider important. The claims are broadly drawn to methods to detect and identify Candida species in a sample by hybridizing the sample with at least one species specific probe selected from (SEQ ID NO: 2-13, 33-38) and detecting the hybridization complexes formed to identify the species present.

The response, filed June 24, 2002, asserts there must be some motivation to slect the claimed species or subgenus in the cited prior art (page 16). This argument has been thoroughly reviewed and not found convincing because the rejection clearly sets forth the motivation to select regions which are dissimilar between two closely related organisms. The ordinary artisan would have been motivated to have selected probes to regions which differ between the nucleic acids of *Candida albicans* and *tropicalis* to facilitate the detection and differentiation of the organisms. The explicit teachings of Botelho provide motivation for "unequivocally distinguishing" between *Candida albicans* and *tropicalis* allow medically diagnostic tools. Therefore, there is specific motivation in the art to select probes which

differentiate between the species and the ordinary artisan would have looked to regions which varied between the sequences to detect the various organisms.

Thus for the reasons above and those already of record, the rejection is maintained.

8. Newly Added Claims 41-45, 47, 49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol. 49, No. 1, pg. M23-M28) in view of Hogan (US Pat. 5,595,874, January 1997).

Williams et al (herein referred to as Williams) teaches an alignment of *Candida albicans*, *Candida tropicalis* and *Candida krusei.* Williams teaches obtaining sequences and retrieving sequences from GenBank and EMBL for alignment using CLUSTAL V suite of programs. SEQ ID NO: 1, 2, 3, 6, 9, 33, 36 are embedded within the ITS1 region (limitations of Claims 37 and 38). The sequences are found in regions of variability. Williams teaches ITS1 and ITS4 and ITS1 and ITS2 as primers used to amplify DNA extracted from Candida isolates and archival tissue (limitations of Claim 26). Moreover Williams teaches the Genbank Accession Numbers for six of the Candida species (L47111, L47112, L47114, L47109, L47113, L47107 and L47108). These Genbank Accession Numbers contain SEQ ID NO: 1-10, 33-37. Williams specifically teaches that "the ability to identify Candida within human tissue provides an opportunity to increase our knowledge of the role of candidal species in disease. This is particularly important for patients with CHC because of the association between this form of candidiasis and the development of oral cancer" (pg. M27).

Williams does not specifically teaches the exact probes and primers of the instant application.

However, Hogan et al. (herein referred to as Hogan) teaches variable regions can be identified by comparative analysis of sequences by using a Sun Microsystem ™ computer for

comparative analysis. The "compiler is capable of manipulating many sequences of data at the same time. Computers of this type and computer programs which may be used or adapted for the purposes are commercially available" (col. 5, lines 50-60). Hogan also teaches the use of specific primers col. 6-7, lines 50-67, lines 1-12, and furthermore provides specific guidance for the selection of primers and probes.

> "Once the variable regions are identified, the sequences are aligned to reveal areas of maximum homology or 'match'. At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.
> First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some others). Second, the stability of the probe:target nucleic acid hybrid should be maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10^{O}C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less preferred. Finally, probes with extensive self complementarity should be avoided."

Therefore, it would have been **prima facie** obvious to one of ordinary skill in the art at the time the invention was made to have designed probes and primers to Candida, as taught by Williams, to the regions of variability found between the two species such that the two species may be differentiated as taught by Hogan. The skilled artisan would have been motivated to have designed probes and primers to detect and/or differentiate these Candida species for the benefit of differentiating the species from one another. Williams specifically teaches that "the ability to identify Candida within human tissue provides an opportunity to increase our knowledge of the role of candidal species in disease. This is particularly important for patients

with CHC because of the association between this form of candidiasis and the development of oral cancer" (pg. M27). Thus, the ordinary artisan would have been specifically motivated to identify and differentiate the Candida species. The knowledge in that art at the time the invention was made was extremely high for aligning sequences and finding regions of variability such that sequence comparison and differentiation between internal spacer regions to identify regions of either variability or conservation was well known and studied. The art teaches an alignment of known sequences, namely *Candida albicans, Candida tropicalis, Candida krusei*, as taught by Williams. Identifying regions of variability between the six species to generate probes which are species specific is taught by Hogan. Within the alignment provided between a select group of the Candida species provided by Williams, the instant probes are within regions of variability. Thus, in view of the teachings in the art, a method for detecting fungal pathogenic species using the probes of SEQ ID NO: 1-3, 6, 9, 33, 36 are obvious.

Response to Arguments

The response traverses the rejection. The response does not specifically address the combination of references of the instant rejection. However, applicant's have broadly argued the 103 rejections as a whole. The examiner has addressed these arguments above. Thus for the reasons above and those already of record, the rejection is maintained.

9. Newly Added Claims 41-48, 50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol. 49, No. 1, pg. M23-M28) and Lin (Genbank Accession Number U10987, March 1996) or Lin et al (J. of Clincial Microbiology, Vol. 33, No. 7, pages 1815-1821, July 1995) or Messner et al (Genbank Accession Number U09325, May 1994) or Williams et al -2(Genbank Accession Number L47108, September 1995) in view of Hogan (US Pat. 5,595,874, January 1997).

Williams et al (herein referred to as Williams) teaches an alignment of *Candida albicans*, *Candida tropicalis* and *Candida krusei*. Williams teaches obtaining sequences and retrieving sequences from GenBank and EMBL for alignment using CLUSTAL V suite of programs. SEQ ID NO: 1, 2, 3, 6, 9, 33, 36 are embedded within the ITS1 region (limitations of Claims 37 and 38). The sequences are found in regions of variability. Williams teaches ITS1 and ITS4 and ITS1 and ITS2 as primers used to amplify DNA extracted from Candida isolates and archival tissue (limitations of Claim 26). Moreover Williams teaches the Genbank Accession Numbers for six of the Candida species (L47111, L47112, L47114, L47109, L47113, L47107 and L47108). These Genbank Accession Numbers contain SEQ ID NO: 1-10, 33-37. Williams specifically teaches that "the ability to identify Candida within human tissue provides an opportunity to increase our knowledge of the role of candidal species in disease. This is particularly important for patients with CHC because of the association between this form of candidiasis and the development of oral cancer" (pg. M27).

Lin et al (herein referred to as Lin) teaches the ITS1 region of Candida parapsilosis which comprises SEQ ID NO: 4 and SEQ ID NO: 5.

Messner et al (herein referred to as Messner) teaches the ITS1 region of Kluyveromyces marxianus which comprises SEQ ID NO: 7 and 8 (anamorph Candida kefyr).

Williams et al -2(herein referred to as Williams-2) teaches the ITS1 of Candida glabrata which comprises SEQ ID NO: 10.

Neither Williams-1, Linn, Messner nor Williams-2 specifically teaches the exact probes and primers of the instant application.

However, Hogan et al. (herein referred to as Hogan) teaches variable regions can be identified by comparative analysis of sequences by using a Sun Microsystem ™ computer for comparative analysis. The "compiler is capable of manipulating many sequences of data at the

same time. Computers of this type and computer programs which may be used or adapted for the purposes are commercially available" (col. 5, lines 50-60). Hogan also teaches the use of specific primers col. 6-7, lines 50-67, lines 1-12, and furthermore provides specific guidance for the selection of primers and probes.

> "Once the variable regions are identified, the sequences are aligned to reveal areas of maximum homology or 'match'. At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.
> First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some others). Second, the stability of the probe:target nucleic acid hybrid should be maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10^{O}C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less preferred. Finally, probes with extensive self complementarity should be avoided."

Therefore, it would have been **prima facie** obvious to one of ordinary skill in the art at the time the invention was made to have designed probes and primers to Candida, as taught by Williams-1, Linn, Messner or Williams-2, to the regions of variability found between the two species such that the two species may be differentiated as taught by Hogan. The skilled artisan would have been motivated to have designed probes and primers to detect and/or differentiate these Candida species for the benefit of differentiating the species from one another. Williams specifically teaches that "the ability to identify Candida within human tissue provides an opportunity to increase our knowledge of the role of candidal species in disease. This is particularly important for patients with CHC because of the association between this form of

candidiasis and the development of oral cancer" (pg. M27). Thus, the ordinary artisan would have been specifically motivated to identify and differentiate the Candida species. The knowledge in that art at the time the invention was made was extremely high for aligning sequences and finding regions of variability such that sequence comparison and differentiation between internal spacer regions to identify regions of either variability or conservation was well known and studied. The art teaches an alignment of known sequences, namely *Candida albicans, Candida tropicalis, Candida krusei*, as taught by Williams. Aligning additional sequences known in the art at the time of the invention would have been routine as provided in Williams and Hogan. Identifying regions of variability between the species to generate probes which are species specific is taught by Hogan. Thus, in view of the teachings in the art, a method for detecting fungal pathogenic species using the probes of SEQ ID NO: 4-5, 7-8, 10 are obvious.

Response to Arguments

The response traverses the rejection. The response does not specifically address the combination of references of the instant rejection. However, applicant's have broadly argued the 103 rejections as a whole. The examiner has addressed these arguments above. Thus for the reasons above and those already of record, the rejection is maintained.

10. Newly added Claims 41-43, 51 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lott et al (US Pat. 6,242,178, June 2001) in view of Hogan (US Pat. 5,595,874, January 1997).

Lott et al (herein referred to as Lott) teaches numerous ITS2 regions from Candida organisms. Lott teaches that the nuclei acid molecules described are useful as probes to detect, identify and distinguish or differentiate between Candida species in a sample or

specimen with high sensitivity and specificity. Lott continues to state that "it will be understood that the probes provided herein are merely exemplary and that those skilled in the art could identify additional portions or fragments of each ITS2 sequence to be used as species-selective probes without undue experimentation from the sequences provided" (col. 5, lines 40-45). Furthermore, the ITS2 region for each Candida species offers a number of very unusual sequences for use as PCR primers. Therefore, comparisons can be made between the Candida ITS2 sequence of two or more species to identify unique or non-homologous regions that would be useful to construct probes that would be specific for distinguishing between those Candida and have minimal cross-hybridization with ITS21 regions from other species. (col. 5, lines 50-60). The specification also provides a computer program for generating selective probes. Lott teaches the ITS2 region of Candida dubliniensis. Probes and primers which are species specific were identified. SEQ ID NO: 12 taught by Lott is the ITS2 region of C. dubliniensis. Namely, SEQ ID NO: 35, a species-specifc probe for Candida dubliniensis overlaps 15 of the nucleotides from the instant SEQ ID NO: 12.

Lott does not specifically teaches the exact probes and primers of the instant application.

However, Hogan et al. (herein referred to as Hogan) teaches variable regions can be identified by comparative analysis of sequences by using a Sun Microsystem ™ computer for comparative analysis. The "compiler is capable of manipulating many sequences of data at the same time. Computers of this type and computer programs which may be used or adapted for the purposes are commercially available" (col. 5, lines 50-60). Hogan also teaches the use of specific primers col. 6-7, lines 50-67, lines 1-12, and furthermore provides specific guidance for the selection of primers and probes.

> "Once the variable regions are identified, the sequences are aligned to reveal areas of maximum homology or 'match'. At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is

recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.
First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some others). Second, the stability of the probe:target nucleic acid hybrid should be maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10^{O}C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less preferred. Finally, probes with extensive self complementarity should be avoided."

Therefore, it would have been **prima facie** obvious to one of ordinary skill in the art at the time the invention was made to have designed probes and primers to Candida, as taught by Lott, to the regions of variability found between the two species such that the two species may be differentiated as taught by Hogan. The skilled artisan would have been motivated to have designed probes and primers to differentiate these Candida species for the benefit of differentiating the species from one another. The ordinary artisan would have been specifically motivated to identify and differentiate the Candida species within the ITS2 region. The knowledge in that art at the time the invention was made was extremely high for aligning sequences and finding regions of variability such that sequence comparison and differentiation between internal spacer regions to identify regions of either variability or conservation was well known and studied. There is a reasonable expectation of success for aligning known sequences, as taught by Lott et al with the *Candida dubliniensis* sequence of Lott using known computer alignment methods and identifying regions of variability between the species to generate probes which are species specific, as taught by Hogan. Within the alignment provided between a select group of the Candida species provided by Lott, the instant probes are

within regions of variability. Thus, in view of the teachings in the art, a method for detecting fungal pathogenic species using the probes of SEQ ID NO: 11-12 are obvious.

Response to Arguments

The response traverses the rejection. The response does not specifically address the combination of references of the instant rejection. However, applicant's have broadly argued the 103 rejections as a whole. The examiner has addressed these arguments above. Thus for the reasons above and those already of record, the rejection is maintained.

11. Claim 52 is rejected under 35 U.S.C. 103(a) as being unpatentable over Botelho et al. (Yeast, Vol. 10, pg. 709-717, 1994) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to Claims 41-43, 45, 47 above further in view of Fujita et al. (J. of Clinical Microbiology, Vol. 33, No. 4, pg. 962-967, April 1995).

12. Claim 52 is rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol. 49, No. 1, pg. M23-M28) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to Claims 41-45, 47, 49 above further in view of Fujita et al. (J. of Clinical Microbiology, Vol. 33, No. 4, pg. 962-967, April 1995).

13. Claim 52 is rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol. 49, No. 1, pg. M23-M28) and Lin (Genbank Accession Number U10987, March 1996) or Lin et al (J. of Clincial Microbiology, Vol. 33, No. 7, pages 1815-1821, July 1995) or Messner et al (Genbank Accession Number U09325, May 1994) or Williams et al (Genbank Accession Number L47108, September 1995) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to claims 41-48, 50 above, and further in view of Fujita et al. (J. of Clinical Microbiology, Vol. 33, No. 4, pg. 962-967, April 1995).

14. Claim 52 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lott et al (US Pat. 6,242,178, June 2001) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to

Claims 41-43, 51 above, and further in view of Fujita et al. (J. of Clinical Microbiology, Vol. 33, No. 4, pg. 962-967, April 1995).

Neither Williams, Botelho, Lin, Messner, Lott, Williams-2 nor Hogan specifically teach detection of fungal species using a solid support.

However, Fujita et al (herein referred to as Fujita) teaches the detection of Candida species in blood using the ITS2 region of the species. Fujita specifically teaches a microtitration plate hybridization assay where digoxigenin- and biotin labeled oligonucleotide probes were detected in an EIA by capture with streptavidin-coated microtitration plates. The microtitration plates were coated with a single-stranded DNA for hybridization studies. As seen in Table 2, a matrix format against DNA from other Candida species as well as from other fungi was used. All probes were tested against all of the target DNAs so that the pattern of reactivity could be detected (pg. 964). Fujita teaches that PCR products were previously detected with Southern blotting or EtBr staining of agarose gels, but these methods are less sensitive that the microtitration plate EIA. Specifically Fujita teaches that microtitration plate EIA detection of C. albicans DNA following PCR is easier and more rapid than that by Souther blotting (pg. 966, col. 1).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified methods of Williams, Botelho, Linn, Messner or Williams-2 or Lott in view of Hogan with the teachings of Fujita. The ordinary artisan would have readily recognized the improvements of solid support detection as taught by Fujita for the detection of PCR amplified DNA. The ordinary artisan would have been motivated to have detected the PCR amplified DNA using a microtitration plate EIA for the express benefits of increased sensitivity, ease and speed, as described by Fujita. Thus, the ordinary artisan would have detected the fungal pathogens using probes from variable regions of the Candida species,

as taught by Williams and Botelho or Lott et al in view of Hogan, on a solid support at taught by Fujita.

Response to Arguments

The response traverses the rejection. The response does not specifically address the combination of references of the instant rejection. However, applicant's have broadly argued the 103 rejections as a whole. The examiner has addressed these arguments above. Thus for the reasons above and those already of record, the rejection is maintained.

15. Claims 54, 56, 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Botelho et al. (Yeast, Vol. 10, pg. 709-717, 1994) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to Claims 41-43, 45, 47 above further in view of Jordan (US Pat. 6,017,699, January 2000).

16. Claims 54-56, 58, 60, 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol. 49, No. 1, pg. M23-M28) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to Claims 41-45, 47, 49 above further in view of Jordan (US Pat. 6,017,699, January 2000).

17. Claims 54-59, 61, 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol. 49, No. 1, pg. M23-M28) and Lin (Genbank Accession Number U10987, March 1996) or Lin et al (J. of Clincial Microbiology, Vol. 33, No. 7, pages 1815-1821, July 1995) or Messner et al (Genbank Accession Number U09325, May 1994) or Williams et al (Genbank Accession Number L47108, September 1995) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to claims 41-48, 50 above, and further in view of Jordan (US Pat. 6,017,699, January 2000).

18. Claims 54-55, 62-63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lott et al (US Pat. 6.242.178, June 2001) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to Claims 41-43, 51 above, and further in view of Jordan (US Pat. 6,017,699, January 2000).

Neither Williams, Botelho, Lin, Messner, Lott, Williams-2 nor Hogan specifically teach multiplex detection of fungal species using a solid support.

However, Jordan teaches five species-specific primers and probes for Candida. Jordan teaches that a multiplex PCR amplification and agarose gel electrophoretic detection (Figure 4). Jordan teaches, in Example IV, three approaches of carrying out detection and/or confirmation of the four species of Candida (col. 16). Within these approaches, Jordan teaches coating a 96 well plate with biotin labeled primer for detection. Jordan teaches significant increase in the level of sensitivity for detecting the Candidal organism (Table 4)" (col. 19). Jordon teaches that "it would bbe more advantageous economically to have more than one species-specific primer pair together in a single PCR reaction, which is why applicant proposed to multiplex PCR approach" (Col. 19, lines 38-40). Jordon teaches a PCR mix which contains primers for amplifying *C. glabrata, C. parapsilosis, C. tropicalis, and krusei* (col. 19, lines 53-55). Jordon teaches using higher annealing temperature for the multiplex PCR amplification reaction to ensure high stringency; primer binding to homologous template only and not that of *C. glabrata, C. parapsilosis, C. tropicalis, and krusei* (Col. 19, lines 60-67). Jordan also teaches that "use of the PCR master mix containing all 3 newly designated species-specific primer pairs resulted in accurate amplification of the predicted sized fragment for the DNA template added" (col. 21, lines 9-20). The multiplex approach to DNA amplification was successful.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified methods of Williams and Botelho Lin, Messner,

Lott, Williams-2 in view of Hogan and further in view of the teachings of Jordan. The ordinary artisan would have readily recognized the improvements of solid support detection as taught by Jordan for the detection of PCR amplified DNA simultaneously. The ordinary artisan would have been motivated to have detected the PCR amplified DNA which has a "significant increase in the level of sensitivity for detecting the candidal organism (Table 4)". Moreover, the ordinary artisan would have been motivated to have designed primers and probes which may be used in a multiplex analysis method because Jordan teaches the benefits of a multiplex analysis to save on reagents and cost. The necessarily amplification parameters for the multiplex analysis has been provided. Jordon teaches forty cycles of amplification, equal concentration (0.25 uM or 0.5 uM) of primers, agarose gel. Thus, the ordinary artisan would have detected the fungal pathogens using probes from variable regions of the Candida species, as taught by Williams and Botelho or Lott in view of Hogan, in a multiplex reaction as taught by Jordan.

Conclusion

19. No claims allowable over the art.

20. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Enewold Goldberg whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Thursday from 7:00AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

J. Goldberg

Jeanine Enewold Goldberg
October 7, 2002

W. Gary Jones
Supervisory Patent Examiner
Technology Center 1600